

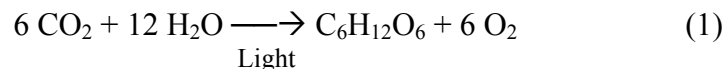
Biology 3A Lab 8 Photosynthesis

Objectives

1. To observe the spectral absorbance of a mixed chlorophyll sample
2. To separate and identify several common plant pigments using paper chromatography
3. To investigate the rate of oxygen production during photosynthesis
4. To measure the effect of wavelength of light on photosynthetic rate
5. To investigate starch production and storage in a single leaf

Introduction

Photosynthesis can be summarized in a fairly simple equation:



This reaction actually occurs in two phases. First, light energy from the sun is captured by the pigment chlorophyll. This first step is often called the light reaction. The next phase does not require light. In this phase carbon (from carbon dioxide) is fixed into glucose. Both of these reactions occur in the chloroplast of plant cells.

In the chloroplast, the chlorophyll is found in the membranes of the grana. When struck by photon of the appropriate wavelength the chlorophyll gives up outer shell electrons to the electron transport chain located in the thylacoid membrane of the chloroplast. The energy in these electrons is used to drive the reaction



by chemiosmosis. In addition high-energy electron are also transferred to NADP, an electron carrier, to create NADPH. The byproduct of this set of reactions is oxygen gas, released from the leaf stomates into the atmosphere.

The ATP and the NADPH produced in the light reaction are used as energy sources to drive reactions that build macromolecules. In these reactions, called the Calvin Cycle (or dark reactions), carbon dioxide is fixed into organic molecules using the energy from ATP and the hydrogen atoms from NADPH. In most plants, the product of the Calvin Cycle is glyceradlhyde-3-phosphate, which is converted into many compounds including glucose and its polymers, starch and cellulose.

Important Note on Timing in This Lab

In this lab several steps require measured time periods. You must set up some procedures, then complete other procedures, and then return to the original procedures. Plan your time carefully. *We expect to complete the entire lab in one period.* Here is a suggested time line for the lab

1. Set up and begin exposure of the photosynthetic “photograph.”
2. Separate plant pigments and set up the paper chromatograms
3. Run the chlorophyll spectral analysis
4. Read the paper chromatograms
5. Develop the photosynthetic “photograph”
6. Set up and run the oxygen production by spinach measurements
7. Set up and run the manometer experiment (effect of wavelength on Ps)

A. Photosynthetic “Photography”

As pointed out above, in the light dependent reactions of photosynthesis, photon energy is absorbed by pigment molecules and transferred to a reactive chlorophyll *a* molecule. In order to illustrate light as a

reactant for photosynthesis, you will use geranium leaves that have been kept in the dark for two days prior to the lab (in order to deplete carbohydrate stores). Photosynthetic rates should vary as a function of light intensity, thus the production and storage of starch should also vary as a function of light. In fact, the rate may vary over the surface of a single leaf, where one part of the leaf is exposed to more light than another! In the macromolecules lab you learned the iodine test for starch. Today we'll apply that method to a whole leaf subjected to different light intensities.

Exposure Procedure:

1. Soak a piece of black cloth in a 0.1M sodium bicarbonate solution.
2. Sandwich 1 leaf with petiole (stalk) and saturated cloth between 2 glass plates making sure the leaf is flat.
3. On the outside of the glass, on the leaf side, place the negative of the photograph. Secure the entire "sandwich" with rubber bands near the edges of the glass plates. Place this on top of a small beaker of lukewarm water so that the petiole at the bottom sits well under the water surface.
4. Use the slide projector to create bright light to expose the image onto the leaf for **1 hour**.

Development Procedure:

1. After at least one hour, carefully remove the leaf from the glass plates. Place the leaf in boiling 70% alcohol for three to five minutes to leach out the pigments. The treated leaf should be brownish-white in color.
2. Place leaf in boiling water for 3 minutes to rinse.
3. Place leaf in a Petri dish and cover with iodine. It takes at least 2 – 3 minutes to develop, turn the leaf over to see the image.

B. Photosynthetic Pigments

Pigments are chemical compounds that reflect specific wavelengths of visible light. This makes them appear "colorful". Leaves, flowers, corals, and even animal skin contain pigments that give them their colors. In addition to reflecting wavelengths, pigments absorb certain wavelengths. This makes pigments useful to plants and other autotrophs. In plants, algae, and cyanobacteria, pigments are the means by which the energy of sunlight is captured for photosynthesis. Since specific pigments absorb only a narrow range of the spectrum, there is usually a need to produce several pigments, each of a different color, to maximize the capture of solar energy.

Three Basic Groups of Plant Pigments: Chlorophylls, Carotenoids and Phycobilins

Chlorophylls are greenish pigments that are used to "capture" the energy of sunlight. There are several kinds of chlorophyll; the most important is chlorophyll *a*. This is the molecule that passes its energized electrons on to molecules that will manufacture sugars. All plants, algae, and cyanobacteria that photosynthesize contain chlorophyll "a". A second kind is chlorophyll *b* occurs only in "green algae" and in the plants. A third form, chlorophyll *c*, is found only in the dinoflagellates. The differences between the chlorophylls of these major groups were used to study their evolutionary relationships.

Carotenoids are usually red, orange, or yellow pigments. There are two major groupings of carotenoids, carotenes (orange color) and xanthophylls (yellow color). These compounds are composed of two small six-carbon rings connected by a "chain" of carbon atoms. As a result, they do not dissolve in water, and must be attached to membranes within the cell. Carotenoids cannot transfer light energy directly to the photosynthetic pathway; they pass their absorbed energy to chlorophyll. For this reason, they are called **accessory pigments**. The familiar β -carotene is found in many plants, and of course is responsible for the orange color of carrots. Of the xanthophylls, lutein and fucoxanthin are

the most common. Lutein is found in all of the large algal forms and fucoxanthin (brown-yellow) is found only in the brown algae (Phaeophyta) and diatoms.

Phycobilins are water-soluble pigments found in the cytoplasm or the stroma of the chloroplast. They occur only in Cyanobacteria and red algae (Rhodophyta). Phycocyanin is a blue pigment, which gives the Cyanobacteria their name ("blue-green algae"). A reddish pigment, phycoerythrin, gives the red algae their common name. The phycobilins are also used as research and medical tools. Both phycocyanin and phycoerythrin fluoresce at specific wavelengths. The light produced by this fluorescence is so distinctive and reliable, that phycobilins may be used as chemical "tags". The pigments are bonded to antibodies, which are then put into a solution of cells. This has found extensive use in cancer research, for "tagging" tumor cells.

Plant Pigment Procedures

B1. Paper Chromatography. We will use paper chromatography to separate the pigments found in several species of plants. The most common, and most easily seen pigment is chlorophyll a, however, we may see some of the more obscure pigments as well.

Run chromatograms on spinach and two other plants or plant parts. To aid in your pigment identification, xanthophylls will appear as a greenish-yellow band, β -carotene should appear as a sharp bright-yellow band, chlorophyll a is blue green and chlorophyll b is yellow green.

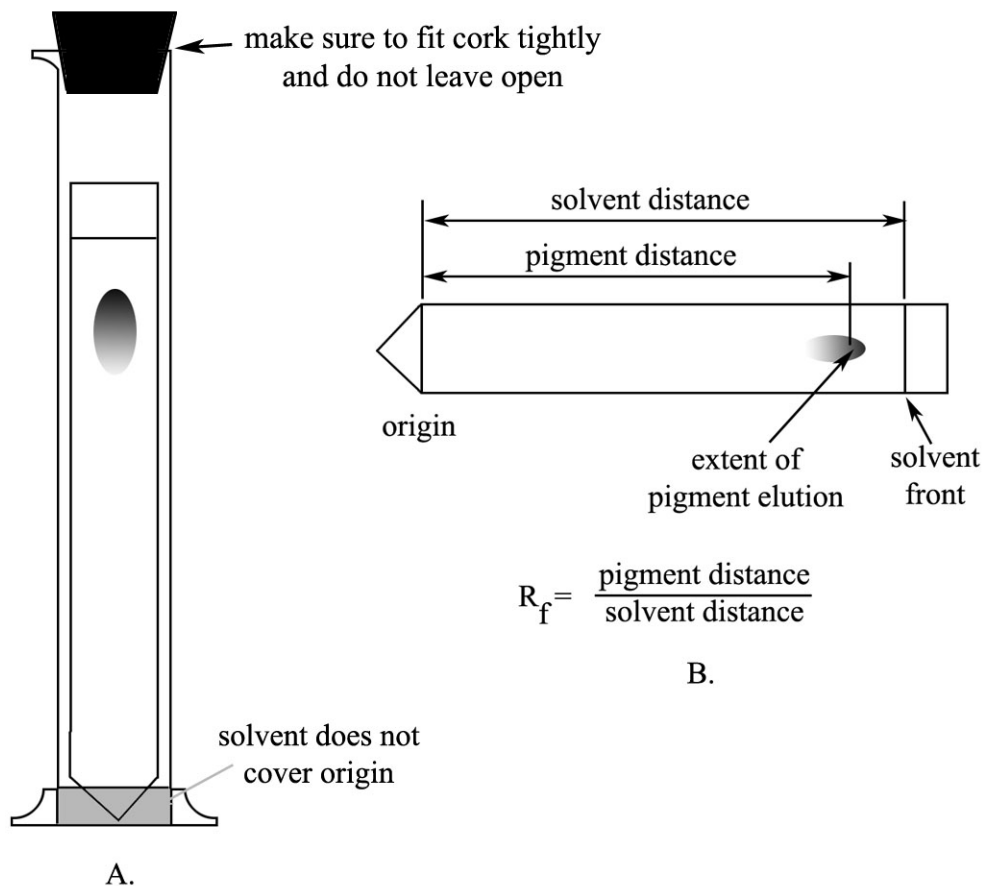


Figure One. Pigment chromatography. A. Paper strip in chromatography chamber. B. Calculation of R_f values.

1. Obtain a strip of chromatography paper. Handle the strip only by the edges. Do not touch the flat surface as hand oils will interfere with the pigment elution.
2. Cut one end into a point.
3. Make a small mark to indicate the origin.
4. Place 10 or so grams of a single species of plant (leaves or other parts) into the mortar. Add a small amount of sand and grind for 30 seconds with the pestle. Transfer a small amount of this material to the bottom (pointed end) of the chromatography paper at the origin.
5. Place the paper into the chromatography chamber (corked graduated cylinder with petroleum ether and acetone) as demonstrated by instructor (see Figure 1.). Replace the cork immediately.
6. Carefully watch the solvent as it progresses up the paper. When the “front” is about 2 cm from the top of the strip, remove the strip and lay it on a paper towel. Replace the cork!
7. Immediately mark, with a pencil, on the strip the extent of the solvent front.
8. Measure the distance from the origin to the extent of the solvent front and the distance from the origin to the center of each color band. Calculate R_f values for each pigment in the sample. Record these data in Figure 3.

B2. Chlorophyll Spectral Analysis

1. In this procedure you will extract the pigment present in spinach and use the spectrophotometer to determine which visible spectrum wavelengths this plant utilizes.
1. Obtain a large fresh spinach leaf and a 50 ml beaker.
2. Rip the leaf into quarters and crush with your hand (avoid over handling the leaf)
3. Place the crushed leaf parts into the beaker and add 20 ml of ethanol (**CAUTION** ethanol is flammable)
4. Place the beaker on a hot plate and heat until the solution acquires a clear green color. **DO NOT BOIL**. Use caution when handling the hot beaker.
5. When the solution is cool, use it to fill 1/4 of a cuvette tube. Fill the remaining 2/3 of the cuvette with ethanol.
6. Set the spectrophotometer on TRANSMITTANCE at 550 nm.
7. Set the spectrophotometer to 100% Transmittance using a “blank” tube filled with only ethanol.
8. Insert your pigment extract into the spectrophotometer and read the transmittance. The reading should be between 65% and 85%. If it is not, adjust the density using more ethanol or more extract until it falls into this range.
9. You are now ready to record your spectral data. Set the spectrophotometer on ABSORBANCE.
10. Set the wavelength to 400 nm. Using the “blank” tube, set the absorbance to zero. Read the absorbance of the pigment extract. Record the absorbance at this wavelength in Table 1.
11. Change the wavelength to the as indicated in Table 1. You must recalibrate with the blank each time you change the wavelength. Read your pigment extract at each of the indicated wavelengths.
12. Using Excel, graph the absorbance spectrum of the spinach extract.

C. Oxygen production in spinach leaf disks

Leaf tissue is filled with intercellular spaces. Gases (oxygen and carbon dioxide) diffuse through these spaces on their way to and from the chloroplasts. As a result of these gas-filled spaces, leaves float. However, if a leaf is placed in a solution and subjected to a vacuum, the gas will be pulled from these spaces and replaced by the solution, causing the leaf to sink. If the leaf is exposed to light, photosynthesis will produce oxygen, which will fill these spaces and cause the leaf to float again. In this experiment, sodium bicarbonate solution will be used to infiltrate the leaf (because it will provide a carbon dioxide source). The time until the leaf disk floats will be a measure of photosynthetic rate.

Procedure

1. Place approximate 100 ml of 0.2% NaHCO₃ into a 250 ml flask. Fill three Petri dishes approximately 2/3 full with the same solution.
2. Cut 40 to 50 leaf disks from fresh spinach using a cork borer against a layer of paper towels. You can stack several leaves together and cut multiple disks. Make sure the disks are not ragged, however.
3. As they are cut, place the disks into the 250 ml flask.
4. Place the cork assembly into the flask and use the vacuum to sink the disks. Note: You will apply the vacuum, then release while swirling the flask. The disks will continue to float while under the vacuum. They will only sink when the vacuum is released. It may take several cycles to sink the disks. It is acceptable if a few disks remain floating
5. Pour the disks and the solution into an evaporating dish. With forceps gently transfer 10 - 15 disks to each Petri dish. Place the lid onto each of the Petri dishes.
6. Place one dish under a 1000 ml beaker of water with a lamp set above. Place one dish into a dark place, such as a cabinet or drawer. Place one dish on the upper lab bench where it will receive only room light.
7. After 10 minutes record how many disks are floating (or turned up standing on edge) in each treatment. After 20 minutes record how many disks are floating? Record your data in Table 2.

D. Wavelength and Photosynthetic Rate

Thus far we have investigated the increase in the rate of photosynthesis as a function of light intensity. However, from our spectral analysis it is clear that wavelength of incident radiation will also effect photosynthetic rate. In this experiment we will estimate the photosynthetic rate by measuring the rate of oxygen production of the plant. It should be noted, however, that this is not an exact measure of photosynthetic rate because some of the oxygen produced used by the plant cells in the process of aerobic respiration.

Procedure:

1. Obtain a piece of *Elodea* 12 cm long and place in a test tube with the cut end up and fill the test tube with 0.1 M NaHCO₃ solution (recall, this is a source of additional CO₂ for the plant).
2. Add a rubber stopper containing a pipette and a syringe (Caution – needles are sharp!) **AVOID** air bubbles under the rubber stopper (Figure 2).
3. Some of the fluid in the test tube will flow into the pipette. The position of the fluid in the pipette can be adjusted by raising and lowering the plunger in the syringe.
4. Prepare a second tube without *Elodea* to act as a thermal barometer. This tube will control for temperature and atmospheric pressure fluctuations.
5. Wrap each tube with one layer of the colored cellophane. There are three colors, your instructor will assign one color to you. Place both tubes in beaker of water at room temperature.
6. Set-up lamp as shown in Figure ? Be sure both tubes are equally exposed to the light source.
7. The test tubes should be about 40 cm from the light source.
8. Allow the *Elodea* in the test tube to stabilize for 5 minutes. Adjust the fluids in the manometers so that they are at the 0.2 ml mark.
9. Read the position of the fluid in the pipettes at 2 minute intervals for 20 minutes.
10. Record your results for experimental and control tubes in Table 3.
11. Your instructor will set up white light and dark control tubes. You may be asked to record data from these as well. Collect all class data. Using Excel, graph the effects of wavelength on photosynthetic rate.
12. Your instructor will provide absorbance spectra for each of the colored cellophanes. Create another graph in which you overlay the chlorophyll spectral absorbance data (from earlier in this lab) with the photosynthetic rates at three different wavelengths.

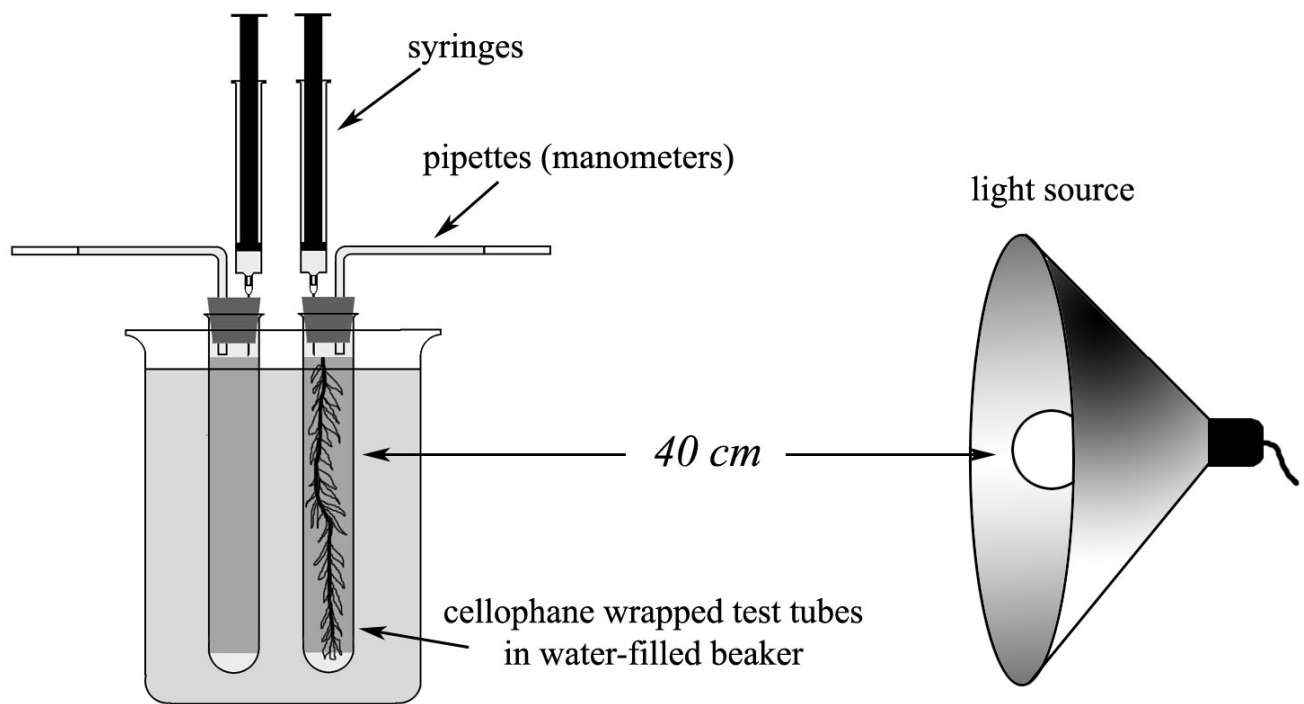


Figure Two. Test tubes in colored cellophane placed in water filled beaker. Light source should be 40 cm from tubes.

Biology 3A Laboratory
Photosynthesis

Name: _____

A. Photosynthetic Photography

1. Do you see the image in the leaf? Is the image a positive or negative of the original? Explain your answer to this question.

B1. Chromatography of Photosynthetic Pigments

Draw the results of your chromatograms

Species _____

Pigments present				
R_f values				

Species _____

Pigments present				
R_f values				

Species _____

Pigments present				
R_f values				

Figure 3. Results of paper chromatography on three plant species

2. How does the number of pigments vary among the three species?

3. How does the type of pigment vary among the species? How can you account for this variation?

B2. Spectral Analysis of Spinach Extract

Complete the following table

Table One. Spectral Absorbance of a Spinach Extract

Wavelength (nm)	Absorbance	Color
400		
420		
440		
460		
480		
500		
520		
540		
560		
580		
600		
620		
640		
660		
680		
700		

4. What colors of light are absorbed by the spinach leaves? What light colors do the spinach leaves transmit? What colors do living spinach leaves reflect?

5. Construct an Excel graph of wavelength vs. absorbance for you extract.

C. Oxygen production during Photosynthesis.

Table 2. Number and percentage of leaf discs floating after 10 and 20 minutes

Light Source	No. Floating at 10 minutes	% Floating at 10 minutes	No. Floating at 20 min	% Floating at 20 minutes
Dark				
Room				
Lamp				

6. State a reasonable hypothesis that was tested in this experiment.

7. Does the data collected support this hypothesis.

8. Construct an Excel graph that would be used as figure to support this hypothesis

9. If you repeated this experiment with using different colors of light (other wavelengths), what would you predict as outcomes for blue, green and red light.

D. Wavelength and Photosynthetic Rate

Table 3. Total oxygen production in ml for *Elodea* exposed to five different light conditions. Corrected oxygen production equals experimental tube minus control tube.

Light Color	Total Oxygen production (ml)						
	Exp Tube 1	Control Tube1	Corrected O ₂ Prod 1	Exp Tube 2	Control Tube2	Corrected O ₂ Prod 2	Mean O ₂ Prod
Dark							
White							
Red							
Green							
Blue							

10. In general, what is the effect of wavelength on the rate of oxygen production?

11. Is the rate of oxygen production an exact measure of total photosynthesis? Explain

12. What variables are not controlled in this experiment? How could you control for these variables?

13. Using the whole class data set (available on the web) create an Excel bar graph that relates light color to oxygen production.

14. First let's express oxygen production as a rate. Divide the mean oxygen production by the total time for the experiment, 20 minutes. What are the units for this rate?

Now, examine the spectral analyses provided for each of the cellophane colors used in this experiment. For each color choose a major transmitted wavelength range.

Finally create an Excel graph in which you show the absorbance spectrum of a typical plant pigment (from exercise B2 above) and the rate of oxygen production vs. wavelength.

Suggestions:

- Wavelength should be on the x-axis

- White light and darkness don't fall on this graph

- Use Oxygen Production in ml/min

- On one y-axis place the absorbance values

- On the other y-axis place the oxygen production values

- You may fit a line to the oxygen production, or use bars (your choice)